Poster Abstract Number: 5520

NXP900, a Phase 1, first-in-class YES1/SRC inhibitor demonstrates potent single agent activity and synergy with ALK inhibitors in ALK resistant NSCLC models

Neil O. Carragher[1], John C Dawson[1], Enrique Poradosu[2], Asier Unciti-Broceta[1]

[1]Edinburgh Cancer Research, CRUK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, UK. [2]Nuvectis Pharma Inc. 1 Bridge Plaza, 2nd Floor, Fort Lee, NJ, 07024, USA.

Introduction

NXP900 (eCF506) is a novel potent and selective SRC family kinase (SFK) inhibitor, (IC50 of 0.47 nM against YES1) which possesses a unique/first-in-class binding mode-of-action. NXP900 locks its target into its native "closed" conformation, thereby inhibiting both kinase activity and scaffolding function with protein signaling partners (Temps et al.). In contrast, multi-kinase inhibitors, including dasatinib and bosutinib, lock SRC in the active "open" conformation (type 1 inhibitors) promoting the association of SFK and signaling partners via allosteric facilitation (Higuchi et al.).

Anaplastic lymphoma kinase (ALK) inhibitors are clinically effective for the treatment of non-small cell lung cancer (NSCLC) patients whose tumors harbor ALK fusion genes. However, the development of resistance to ALK inhibitors in patients is nearly universal. Activation of SFK signaling has previously been shown to mediate acquired resistance to ALK inhibition in NSCLC lines (Yoshida et al. Sato et al.) indicating a potential for NXP900 to overcome an important bypass resistance mechanism and offer therapeutic potential in cancers with acquired resistance to ALK inhibitors.

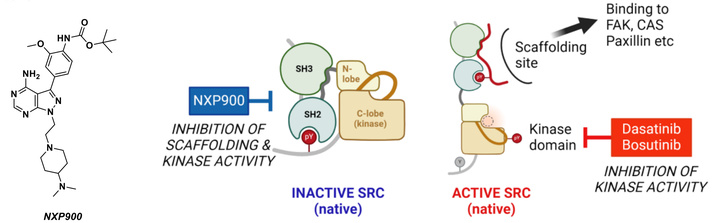


Figure 1. Mechanism of SRC inhibition. NXP900 locks SRC in its native inactive conformation inhibiting both catalytic and scaffolding functions (Created with Biorender).

Methods

Resistant NSCLC cell lines to ALK inhibitors were generated from NCI-H2228 (EML4-ALKv3) and NCI-H3122 (EML4-ALKv1) following exposure to increasing concentrations of alectinib or crizotinib.

Cell proliferation assay - Cells were dispensed in a 384-well plate, for each cell line the optimal cell density is used, cells were treated with ALK inhibitors alectinib, lorlatinib and/or NXP900 for 120 h and underwent ATPlite viability assay (Oncolines B.V., The Netherlands). Drug combination effects in vitro were studied using the SynergyFinder+ R package applied across multiple fixed dose ratio mixtures (Zheng et al.).

NXP900 potently inhibits SRC autophosphorylation in ALK inhibitor sensitive and resistant NSCLC cell lines

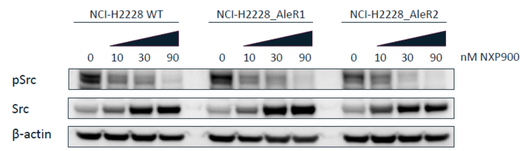


Figure 1. Phospho-Src (Tyr419) is inhibited at low nM concentrations of NXP900 for both parental and alectinib-resistant cell lines.

NXP900 inhibits proliferation of cell lines with acquired resistance to ALK inhibitors

- **Acquired resistance to ALK inhibitors results in increased sensitivity to SRC/YES1 inhibition.**
- **Acquired resistance to either alectinib or crizotinib induces cross-resistance to other ALK inhibitors.**
- **No secondary mutations in the ALK-fusion gene were identified in independently generated resistant clones.**

Cell line	Alectinib	Lorlatinib	Crizotinib	NXP900
NCI-H3122	364	1.9	318.2	14,347
NCI-H3122_CriR1	777	3.1	839.8	73
NCI-H3122_CriR3	2,101	47	2,165.1	56
NCI-H2228	76	1.3	234	82
NCI-H2228_AleR1	6460	801	1,173	26
NCI-H2228_AleR2	6614	172	1,295	19
NCI-H2228_AleR3	2,962	421	982	30
NCI-H2228_AleR5	2,158	323	1,226	22
NCI-H2228_AleR14	N.A.	8,583	1,873	44
NCI-H2228_AleR20	N.A.	5,141	2,804	30

Table 1. Cell proliferation assay: Cells were dispensed in a 384-well plate, for each cell line the optimal cell density is used. Cells were treated with the indicated inhibitors for 120 h. Data shown represents GI50 (nM).

NXP900 demonstrates potent synergy with ALK inhibitors in EML4-ALKv3 driven, alectinib/lorlatinib/crizotinib-resistant NSCLC cell lines

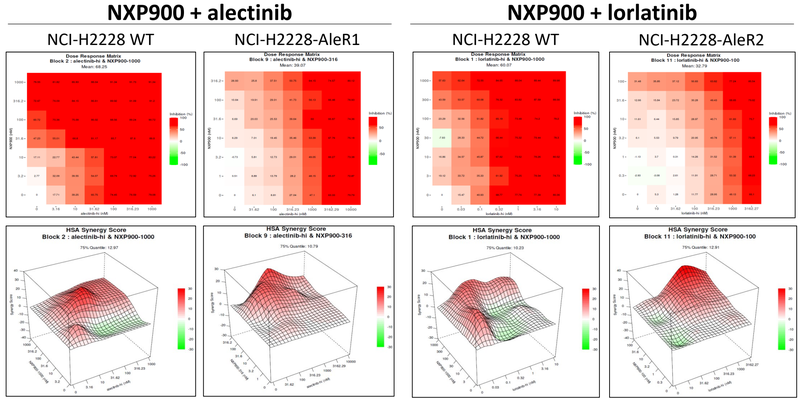


Figure 2. NXP900+alectinib/+lorlatinib dose-ratio matrix combination analysis (Synergy Finder+) across parental (NCI-H2228) and ALK inhibitor (NCI-H2228-ALeR1, -AleR2) resistant cell lines.

NXP900 demonstrates potent synergy with lorlatinib in EML4-ALKv1 driven, alectinib/lorlatinib/crizotinib-resistant NSCLC cell lines

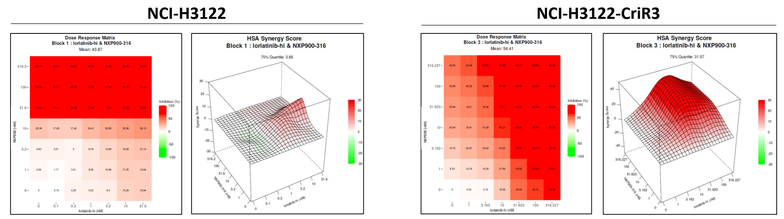


Figure 3. NXP900 + lorlatinib dose-ratio matrix combination analysis (Synergy Finder+) across parental and crizotinib resistant (CriR3) NCI-H3122 cell lines.

Conclusions

- Despite high response rates and long duration of response to ALK inhibitors, acquired resistance almost universally arises.
- Here we demonstrate that NXP900 potently inhibited the activating SRC autophosphorylation across the same concentration range required to inhibit cell proliferation of ALK resistant cell lines as a single agent.
- Inhibition of SRC/YES1 by NXP900 is strongly synergistic in combination with both alectinib and lorlatinib in NSCLC models harboring different variants of the ALK gene fusion that are resistant to alectinib, lorlatinib and crizotinib.
- Altogether, these data suggest that NXP900, in combination with ALK inhibitors, may have therapeutic potential in cancers with acquired resistance to ALK inhibitors.
- A first-in-human, Phase1 dose escalation study for NXP900 is ongoing.

Acknowledgements and Contacts

With thanks to a research grant to the University of Edinburgh provided by Nuvectis Pharma, Inc.

- Asier Unciti-Broceta, University of Edinburgh: asier.ub@ed.ac.uk
- Neil Carragher, University of Edinburgh: n.carragher@ed.ac.uk
- Enrique Poradosu, Nuvectis Pharma Inc.: eporadosu@nuvectis.com

References

1. Temps et. al., Cancer Res. 2021 Nov (21):5438-5450. doi: 10.1158/0008-5472.CAN-21-0613.
2. Higuchi et al., Cell Rep. 2021 Mar 23;34(12):108876. doi: 10.1016/j.celrep.2021.108876.
3. Yoshida et al., Int J Oncol. 2017 Nov;51(5):1533-1540. doi: 10.3892/ijo.2017.4140
4. Sato et al., JCO Precis Oncol. 2022 Aug;6:e2200088. doi: 10.1200/PO.22.00088
5. Zheng et al., Genomics Proteomics Bioinformatics. 2022 20(3):587-596.doi: 10.1016/j.gpb.2022.01.004

© POSTER TEMPLATE BY GENOGRAPHICS® 1-800-790-5001 WWW.GENOGRAPHICS.COM